A STUDY OF THE GERM CELLS OF CORYMORPHA PALMA.

by

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A thesis submitted to the Department of Zoölogy
and the Faculty of the Graduate School
in partial fulfillment of the requirements for the Master's degree.

__________ Department of __________
Zoology
Bennet M. Allen.

June the First
Nineteen Fifteen.

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INTRODUCTION.

The problem which is here described was undertaken at the suggestion of Doctor Bennet M. Allen, to whom I am indebted for assistance and guidance throughout the entire extent of the work. Originally, the problem was to discover the origin and path of migration of the germ cells of Corymorpha, but, as observations accumulated, it has enlarged itself to include the formation and development of the medusa, particularly the formation of that part called, by Weismann, the "glockenkern", which has not been worked out in any detail before. While the problem of the origin is not completely settled here, the germ cells have been traced to a point, beyond which they cannot be followed, owing to the limitations of the material at hand, and the methods of technique.

LITERATURE.

A vast amount of work has been done upon the germ cells of Coelenterates. An excellent review of the results and interpretations of various workers has been given by Hegner in his recent book on "The Germ-cell Cycle in Animals", so it will be unnecessary to devote any space here to the discussion of the general problem.

While much has been done with the germ cells of other hydroids, comparatively few workers have taken up Corymorpha. Weismann observed only a single female medusa of Corymorpha, but describes this form in his "Die Entstehung der Sexualzellen bei den Hydromedusen" (1883), and gives a drawing to show ova in the ectoderm of the manubrium. He finds the older ova in the manubrium and describes germ cells similar to the young ova among the ectodermal cells in the neck of the manubrium. He finds the entoderm to contain no cells that could be called germ cells and concludes that the germ cells arise in the ectoderm. Although he observed no male medusa, he draws the conclusion that the male germ cells must arise in the same way as the female cells in Corymorpha, since they do in other allied forms.

May* has described the morphology and development of

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* May, Albert J. 1903. A contribution to the Morphology and Development of Corymorpha pendula. The American Naturalist, Vol. 37, No. 441.

Corymorpha pendula, the eastern species, which differs from Corymorpha palma, the western species, in only a few minor points. He describes the general morphology of the form, and takes up the development of the medusa. He finds the medusa buds beginning as simple evaginations of the wall of the peduncle. "By a proliferation of ectodermal cells at the distal end of the bud, a plug of ectodermal cells is formed, which grows down into the medusoid cavity forcing back the entodermal cells as it advances." He states that these cells of ectodermal origin give rise to the future reproductive elements, but fails to show how and when definite germ cells arise, and does not trace them through the early stages of development. As the ova develop, certain cells are said to develop at the expense of others, by a process of absorption through pseudopodia-like processes and by the breaking down of cell boundaries, forming a syncytium of cells in which disintegrating nuclei may be seen.

Investigations by Torrey* indicate a third process, the incorporation of cells which have previously begun to disintegrate. Torrey, in speaking of Corymorpha palma, says

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* Torrey, Harry Beal. 1907. Biological Studies on Corymorpha II Development of C. palma from the Egg. University of California Publications. Zoölogy. Vol. 3, No. 12

the germ cells arise in the ectoderm of the manubrium from cells which have been derived from the ectodermal plug, at the apex of the medusa bud. Torrey's other papers on Corymorpha*, fail to throw any additional light upon the subject of the origin or migration of the germ cells.

MATERIAL AND TECHNIQUE.

The material from which these observations were made was collected at Pacific Beach, California, during the summer of 1914. Some of the material was fixed in Flemming's fixative, some in Telleyesniczky's fluid, and some in Zenker's. All of these fixatives gave good results, but, on the whole, the Flemming material was mostsatisfactory.

At first single medusae were cut longitudinally and transversely and an attempt was made to isolate and section in a definite plane small pieces of the peduncle, bearing one or two of the younger buds, in hopes of getting all stages of development, but it has proven more satisfactory to use

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Torrey, Harry Beal.
1902. Hydroida of the Pacific Coast of North America. University of California Publications. Zoölogy, Vol.I, No.I.
1907. Biological Studies on Corymorpha.
I.C. Palma and Environment.
Journal of Experimental Zoölogy, Vol.I., No.3.

the whole peduncle with all but the largest medusae attached. By this method, there is always the chance of cutting a few of the buds in the proper plane, and the sex of the older medusae serves as a guide to the sex of the younger buds, since medusae of only one sex develop on a single peduncle.

The sections were all cut four or five micra in thickness. Benda's Mitochondria stain, Rubaschkin's stain, Bensley's neutral gentian stain, and iron-haematoxylin with eosin or Congo red were used in staining the sections. Iron haematoxylin with neutral gentian as a counter-stain proved the best for differentiating germ cells and the combination of these two stains was used for most of the sections.

I.- FORMATION AND DEVELOPMENT OF THE MEDUSA.

Young medusae of Corymorpha palma usually develop in groups as outpushings from the stalks of older buds, although they frequently occur singly along the main peduncle. The first indication of the formation of a new bud is a clumping of entodermal cells and a rounding out of the ectoderm in this region. (Figure 2). At the time of this outpushing, the nematocyst cells have practically disappeared from the ectoderm of the bud, while the ectoderm and mesoglea both are noticeably thinner here than in other regions of the peduncle (Figure I). This stretching and thinning of ectoderm and mesoglea continues as development proceeds (Figure 3).

Further development of the medusa is shown in figure 3. The bud has elongated and there is a decided thickening of the ectoderm at its apex. The cells here are crowded together and the cytoplasm has rounded up around the nucleus so as to give a dense appearance to the whole region. The ectoderm of the sides and base of the bud consists of a single layer of cells. The cells of the entoderm have come to lie in a row just beneath the mesoglea, those at the tip being more definitely organized than the others farther back. A loose mass of structureless cytoplasm occupies the center of the bud, and a cavity is forming just beneath the well organized cells at the apex.

The next stage (Figure 4) shows the origin of a structure to which Weismann applied the term "glockenkern". Weismann says "Als Entocodon oder Glockenkern bezeichne ich jenes wichtige Embryonalorgan, durch dessen Vermittlung die einfach blindsackförmige Knospe zur Meduse umgewandelt wird, jene schon von so vielen Autoren gesehene und unter verschiedenen Bezeichnungen beschriebene Wucherung des Ektoderms in der Spitze der Knospe, welche den Entodermschlauch eindrückt und so zur Bildung einer becher - oder kelch - förmigen hohlen Entoderm - Duplikatur Anlass giebt, aus welcher die Entodermlage der Medusenglocke hervorgeht. Es is nötig, dafür eine bestimmte, einfache und unzweideutige Bezeichnung zu haben."* This "glockenkern" originates from the thickened mass of ectodermal cells at the tip of the bud. The ectoderm and entoderm have separated in this region and some of the ectoderm cells have passed into the cavity formed by this separation. The "glockenkern" cell mass thus formed, later gives rise to the subumbrella and the ectoderm of the manubrium, which functions as a germ gland. As this "glockenkern" cell mass enlarges, it pushes back the layer of entoderm cells beneath it.

Figure 5 shows the further development of the "glockenkern". The whole bud has increased in size and the pear-shaped cell mass has grown down into the central cavity of the bud, pushing back the endoderm as it advances. Germ cells are first seen in the "glockenkern" at this stage, and

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* Weismann, August.
I883. Die Entstehung der Sexualzellen bei den Hydromedusen.

there is a tendency for the cells to become arranged in two layers, a deeper layer next to the entoderm, containing the germ cells and an outer layer, composed only of somatic cells.

A later stage in the development of the medusa bud is shown in Figure 6. There is a continued increase in size, and the "glockenkern" has spread out to form an inverted cup-shaped mass around a central elevation of entodermal cells. This central elevation indicates the general shape of the manubrium, and that part around the outside, where the entoderm turns sharply back upon itself, will form the umbrella of the medusa. The cells of the "glockenkern" have now separated into two distinct layers. The outer layer, composed only of somatic cells, lies closely pressed against the ectodermal layer surrounding the whole bud. The inner layer, composed of some somatic cells and all of the germ cells which have migrated into the "glockenkern" at this time, lies close to the entodermal cells beneath. This deeper layer is the ectoderm of the manubrium which serves as a germ gland in Corymorpha. The outer layer of somatic cells becomes the lining of the umbrella.

As the medusa bud develops, the split between the two layers of cells in the "glockenkern" completely separates the manubrium from the outer capsule of the bud (Figure 7). At the tip of the bud, this outer capsule is composed of only two layers of cells. It is at this point that the developing manubrium later breaks through the surrounding capsule. Around the sides of the manubrium in the region of the umbrella, the capsule is composed of four layers of cells, two layers of entoderm, formed by one continuous layer folded back upon itself, an outer layer of ectoderm and an inner layer of ectoderm which has been derived from the "glockenkern" call mass.

The cavity of the manubrium and the cavity of the umbrella are both continuations of the central cavity of the bud, which, in turn, communicates with the cavity of the peduncle. A cross section of a bud in this stage, taken through the manubrium and the umbrella (Figure 8) reveals the fact that the cavity of the umbrella is not uniform, but shows clearly the formation of the four radial canals. The germ gland rounds out to form four ridges down the sides of the manubrium, which press together the entoderm in the corresponding regions of the umbrella. In the intermediate regions between the ridges of the manubrium, the entodermal cell layers are widely separated, forming the four radial canals.

As the medusa bud grows, the manubrium increases in size until it finally breaks through the outer capsule at the tip where there are but two layers of cells (Figure 9). Further development of the bud involves no radical change in the arrangement of cell layers. The tips of the radial canals widen out and meet to form the circular canal. The apex of the manubrium never opens to the exterior. The sexes are separate, and the germ cells, either eggs or sperm, are developed in the ectoderm of the manubrium and shed, when ripe, while the medusa is still attached.

In the fully developed medusae there is a distinct difference between the sexes. The male medusa has a relatively longer manubrium and shorter umbrella than the female medusa, and the manubrium of the male is more blunt at the tip than that of the female.

Sexual differentiation is not apparent until fairly late stages of development, but the sex of even the very earliest buds may be determined by their relation to the older medusae in which the sex is evident. Since medusae of only one sex are found on a single peduncle, the sex of young buds will always correspond to that of the older medusae borne upon the same peduncle.

II. THE ORIGIN, MIGRATION AND DEVELOPMENT OF THE GERM CELLS.

I.- Distinguishing characters of early germ cells. The earliest germ cells to be found in Corymorpha palma are marked by certain characters which serve to distinguish them from other cells, entodermal or ectodermal. The nucleus of a primitive germ cell, wherever it may be found, is usually larger than the nuclei of other cells, but this difference in size does not always serve as a means of identification. The nucleolus is large and prominent. The nucleus is much less dense than the surrounding cytoplasm and the deeply staining chromatin is condensed into coarse masses which lie around the periphery, close to the nuclear wall. The combination of these two characters, the clearness of the nucleus, and the arrangement of the chromatin, gives to the nucleus a hollow, rounded appearance which is absolutely distinctive and serves as the safest means of identification. The cytoplasm of the primitive germ cell is usually more dense than that of other cells and contains coarse, deeply staining granules, probably mitochondria. The limits of the primitive germ cells are usually quite definite, marking off the germ cells from other cells among which they are found.

2.- Origin of the Germ Cells.

Primitive germ cells appear scattered throughout all parts of the ectoderm of the peduncle, but are most numerous near the bases of older medusae in regions where new buds are developing (Figure I).

3.- Migration of the Germ Cells.

In order to follow the path of the migrating germ cells, it has been necessary to arbitrarily establish certain stages in the development of the medusa bud. These stages are based entirely upon differences in structure. Since all of the material used for these observations was fixed material, there is absolutely no way of measuring the length of time involved in the development of the successive stages.

Stage A includes all buds from the merest outpushing up to Stage B (Figure 2).

Stage B includes only the elongated buds with a distinct thickening of the ectoderm at the apex (Figure 3).

Stage C includes those buds having the undifferentiated "glöckenkern" cell mass between the ectoderm and entoderm at the tip of the bud (Figure 4).

Stage D includes all buds having the pear-shaped "glockenkern" (Figure 5).

Stage E includes buds similar to the one shown in Figure 6.

Primitive germ cells are found scattered throughout the ectoderm of the peduncle, but are not seen among the ordinary entoderm cells. In all cases where there is any indication of the formation of a medusa bud and in all later stages, germ cells are found among the ectoderm cells of the young bud. Whether the presence of germ cells serves as a stimulus for the development of the bud, or whether the development of the bud attracts the germ cells, cannot be determined, but the presence of germ cells in the entoderm of the earliest buds cannot be denied. The fact that germ cells are normally present in the ectoderm, and are not present in the entoderm, except in the region of developing buds, is a strong indication of migration from ectoderm to entoderm.

In counting the germ cells in each bud, not only the number but the position of the cells has been taken into consideration in order to determine the migration. Some of the larger buds in Stage A and all of the buds in later stages were divided into three parts, proximal, central and distal, and the germ cells were counted in the entoderm and ectoderm of each of these regions. The average of at least two counts was taken as the final number for each bud.

TABLE I.

		ENTODERM			TOTAL	ECTODERM			TOTAL	
BUD	SEX	Prox	Cent	Dist	ENTODERM	Prox	Cent	Dist	ECTODERM	TOTAL
I	?				I6				8	24
2	?				I2				9	2I
3	?				I4				7	2I
4	Male				9				6	I5
5	Male	4	9	4	I7	I0	4	I	I5	32
6	Male	4	7	I	I2	7	2		9	2I
7	Male	5	9	I	I5	3	2		5	20

Table I shows the number of germ cells in Stage A medusa buds. Buds I, 2, 3, and 4 were the youngest buds in which germ cells were counted, and were too small to divide into different regions, but the presence of a considerable number of germ cells in the entoderm at this time and the presence of others in the ectoderm of the peduncle at the sides of the buds indicates the early migration of the germ cells toward the bud. The relative number of germ cells in the different regions of the older buds 5,6, and 7, presents a fairly good indication of the path of migration, followed by the germ cells. The predominence of germ cells in the proximal third of the ectoderm and the predominence of germ cells in the central portion of the entoderm seems to show the passage of such cells from ectoderm to entoderm at a point somewhere within the limits of these two regions. The path of migration, then, is probably from ectoderm to entoderm at the base of the bud and then among the entoderm cells toward the apex. The germ cells in the distal part of the ectoderm are probably abnormal in position, since there are so few, and since there is no indication of a continued migration in that direction.

Allowance must be made for individual variation in the matter of migration. Some medusae show an early and a rapid migration of germ cells, while others show the process delayed. There is apparently no constant difference between the sexes as to this question of migration, nor with regard to the number of primitive germ cells in the early stages.

TABLE II.

NUMBER OF GERM CELLS IN STAGE B BUDS.

BUD	SEX	ENTODERM Prox	Cent	Distal	TOTAL ENTODERM	ECTODERM	TOTAL
I	Male	7	I7	4	28	I	29
2	?	4	8	5	I7	5	22
3	Male	I2	9	I	22	7	29
4	Female	7	8	2	I7	6	23

Table II gives the number of germ cells in Stage B. In this stage, there is an increase in the number of germ cells in the entoderm, accompanied by a corresponding decrease in the number of germ cells in the ectoderm of the bud, without any decided change in the total number as compared with the total number in the previous stage.

TABLE III.

NUMBER OF GERM CELLS IN STAGE C BUDS.

BUD	SEX	ENTODERM Prox	Cent	Distal	TOTAL ENTODERM	ECTODERM	TOTAL
I	?	7	I4		2I	I	22
2	?	3	7	5	I5	2	I7
3	Female	4	7	3	I4	4	I8
4	Male	5	II		I6	3	I9
5	Male	8	II	I	20	6	26

Table III shows the number of germ cells in the medusae at Stage C, the time of the formation of the "glockenkern". There is no great change in the total number of germ cells, but the arrangement is somewhat different. The reduction in the number of germ cells in the ectoderm shows that they are still passing from ectoderm to entoderm without being replaced by other germ cells from the adjacent ectoderm of the peduncle. The continued predominence of germ cells in the central third of the entoderm, and in increase in the number in the distal third indicates a constant migration toward the "glockenkern" at the apex.

TABLE IV.

NUMBER OF GERM CELLS IN STAGE D BUDS

BUD	SEX	ENTODERM Prox	Cent	Dist	TOTAL ENTODERM	ECTODERM	"Glocken-kern"	TOTAL
I	?	6	II	I	I8	3	4	25
2	Female	2	3	3	8		II	I9
3	Female	8	I3	3	24	I		25
4	Female	3	IO	7	20	2		22
5	Female	3	7	3	I3	I	7	2I

In Stage D (Table IV) some germ cells have passed into the "glockenkern" and the germ cells have practically disappeared from the ectoderm without any marked change in the total number. The fact that in the two buds,3 and 4,no germ cells have migrated into the "glockenkern" probably indicates nothing more than an individual variation in the form of a delayed migration.

TABLE V.

NUMBER OF GERM CELLS IN STAGE E BUDS.

BUD	SEX	Prox	Cent	Dist	TOTAL ENTODERM	ECTODERM	Glocken-kern"	TOTAL
I	Male			3	3		25	28
2	Female		I	2	3	2	22	27
3	Male		I	3	4		I9	23
4	Male						32	32
5	Male		I		I		25	26
6	Male		2	7	9	2	I3	24

In Stage E, shown in Table V, a large majority of the germ cells, and, in some cases, practically all of the germ cells, have passed into the "glockenkern". The germ cells still in the entoderm are in a position to migrate later into the "glockenkern" and will probably do so.

4.- Multiplication of Germ Cells and Sexual Differentiation.

Throughout the development of the medusae thus far there has been no apparent increase in the total number of germ cells, and no visible sexual differentiation. After Stage A, there is a multiplication of germ cells in the "glockenkern" and a differentiation of the cells into eggs and sperm in the different medusae.

The egg cells increase enormously in size, the cytoplasm becomes somewhat vacuolated and yolk granules are deposited in it. There is no indication of the actual engulfment of one cell by another, nor the absorption of disintegrating cells by others (Figure IO).

In older male medusae, the number of sperm is considerably greater than the number of eggs in the manubrium of female medusae of corresponding size. The complete process of spermatogenesis can be observed in a single bud (Figure II). The germ cells are rapidly dividing in the region near the entoderm, and later stages are seen near the periphery. The process of differentiation results in a condensation of cytoplasm and nucleus, forming an almost spherical dark body, the sperm. No attempt was made to follow the details of this process.

III.- SUMMARY AND CONCLUSIONS.

I.- The medusa bud begins development as an outpushing of the wall of the peduncle, usually near the base of older medusae.

2.- The "glockenkern" arises as a mass of ectodermal cells pushed into a cavity at the apex of the bud, formed by the separation of the ectoderm and entoderm.

3.- The "glockenkern" cell mass separates into two cup-shaped layers, one going to form the inner lining of the umbrella, the other, containing germ cells, going to form the ectoderm of the manubrium.

4.- As these two layers of the "glockenkern" cell mass push back the entoderm, the manubrium becomes separated from the outer capsule (umbrella) of the bud.

5.- The manubrium breaks through the outer capsule leaving the umbrella free.

6.- Medusae of only one sex are found on a single peduncle, thus making possible the determination of the sex of very young buds.

7.- Primitive germ cells, identified by certain definite characters, are found scattered among the ectodermal cells of the peduncle, most frequently near the base of older medusae.

8.- The presence of germ cells in the entoderm,only in the region of developing buds, indicates the early migration of some germ cells from ectoderm to entoderm at that point.

9.- The path of migration taken by the germ cells is from ectoderm to entoderm at the base of developing buds, then among the entoderm cells and into the "glockenkern" at the apex of the bud. The entrance of the germ cells into the "glockenkern" does not take place until after the latter is well formed.

IO.- The number of primitive germ cells entering into the formation of a single medusa is fairly constant, varying from I7 to 32 in all the medusae counted.

II.- Multiplication of germ cells and sexual differentiation does not occur until a late stage of development.

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ABBREVIATIONS FOR ALL FIGURES.

B. Ent,- Bud entoderm.
Cav.Man.,- Cavity of the manubrium.
Cav.Med.,- Cavity of the medusa.
E.,- Egg.
Ect.,- Ectoderm.
Ect. Th.,- Ectodermal thickening.
Ent.,- Entoderm.
Ent. Man.,- Entoderm of the manubrium.
Ent. Umb.,- Entoderm of the umbrella.
Ext. Ect. Umb.,- External ectodermal layer, of the umbrella.
Ext. Glk.,- External layer of the "glockenkern".
Int. Ect. Umb.,- Internal ectodermal layer of the umbrella.
Int. Glk.,- Internal layer of the "glockenkern".
G.C.,- Germ cell.
Mes.,- Mesoglea.
Nt.,- Nematocyst cell.
R.C.,- Radial canal.
S.C.,- Somatic cell.
Spm.,- Sperm.

PLATE I.

FIGURE I.- Longitudinal section through the ectoderm, entoderm and mesoglea of the peduncle at the base of a bud, showing the germ cells in the ectoderm migrating toward the bud.

FIGURE 2.-Transverse section through the peduncle in the region of a developing bud, showing germ cells in the entoderm, Stage A.

FIGURE 3.-Median longitudinal section through a medusa bud, showing germ cells in the entoderm and the thickening of the ectoderm at the apex. Stage B.

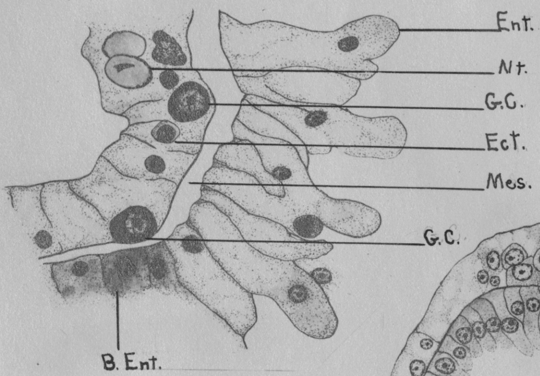
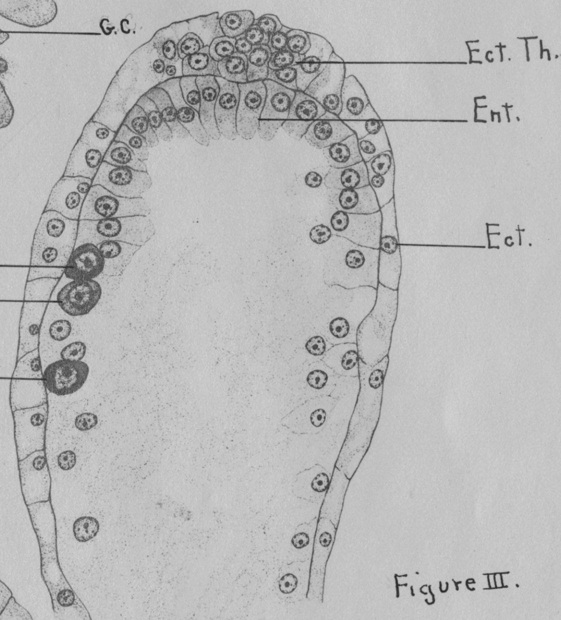


Figure I.



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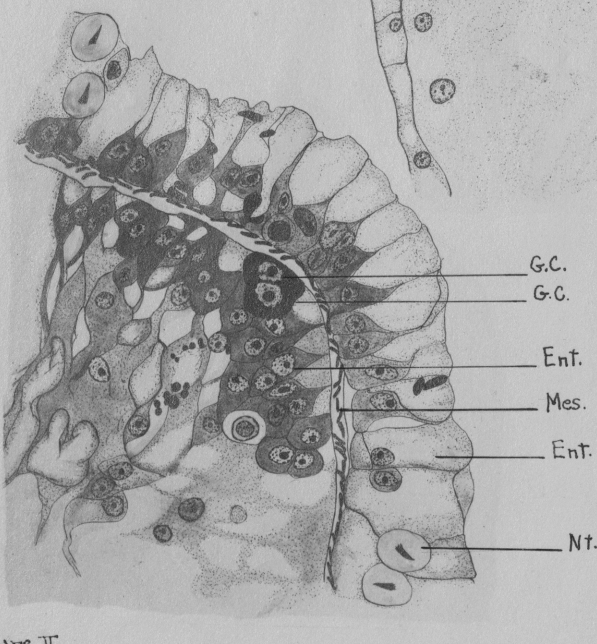


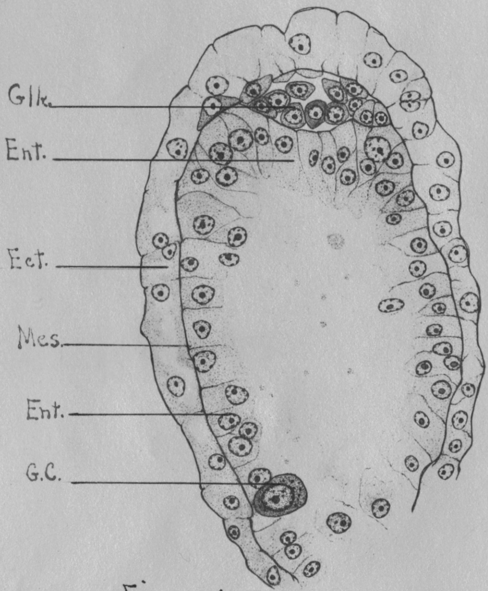
Figure II

PLATE II.

FIGURE 4.- Median longitudinal section through a bud, showing the formation of the "glockenkern" at the apex. Stage C.

FIGURE 5.- Median longitudinal section through a bud, showing germ cells migrating into the pear-shaped "glockenkern". Stage D.

FIGURE 6.- Median longitudinal section through a bud, showing the separation of the two layers of the "glockenkern" cell mass and the formation of the manubrium. Stage E.



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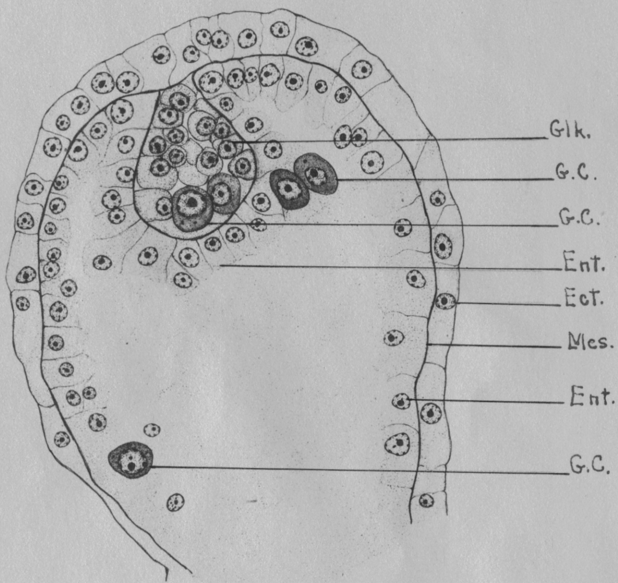

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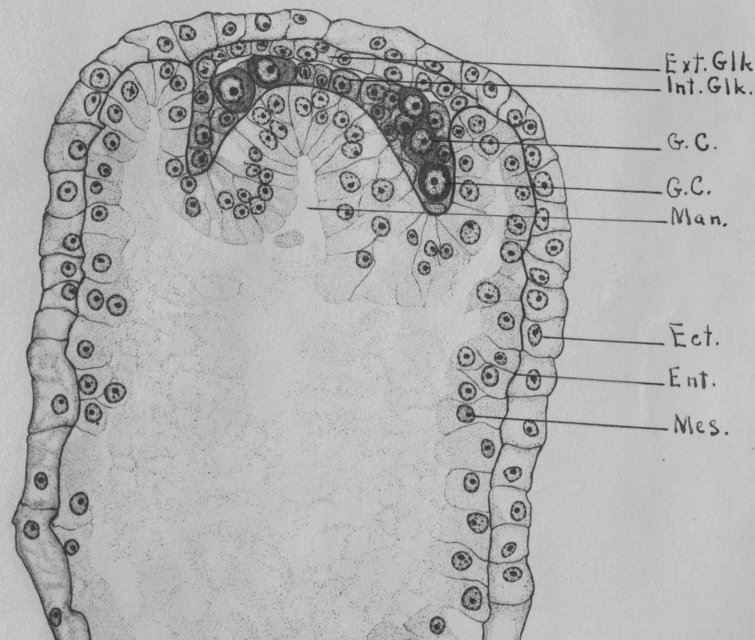


Figure 6.

PLATE III.

FIGURE 7.- Semi-diagrammatic median longitudinal section through a bud, showing complete differentiation of all the cell layers.

FIGURE 8.- Semi-diagrammatic transverse section through a medusa at the same stage of development as the one shown in Figure 7, showing the cell layers and the radial canals.

FIGURE 9.- Semi-diagrammatic median longitudinal section through a medusa, showing the manubrium breaking through the outer capsule.

FIGURE IO.-Segment of a transverse section through the manubrium of an older female medusa.

FIGURE II.-Segment of a transverse section through the manubrium of a male medusa, with a diameter equal to that of the medusa shown in Figure IO.

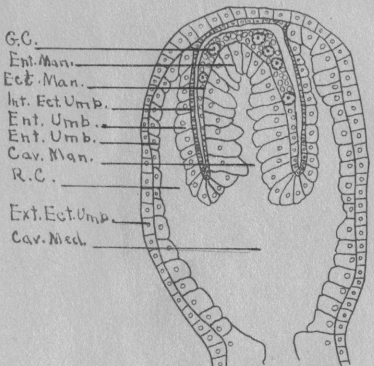


Figure 7.

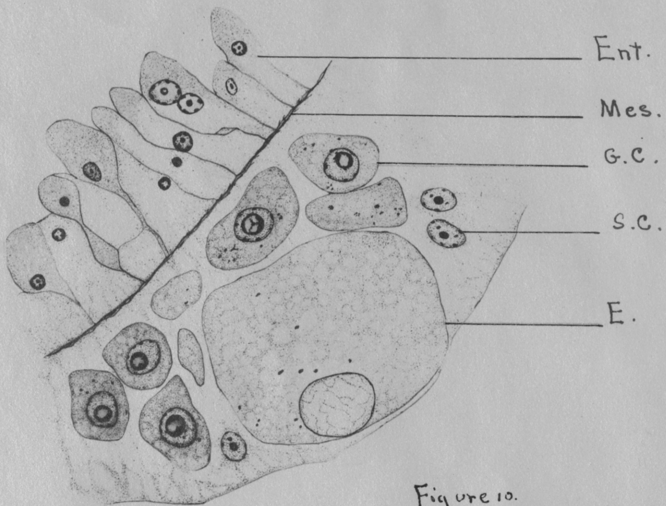
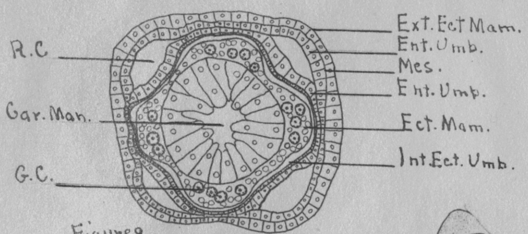


Figure 10.



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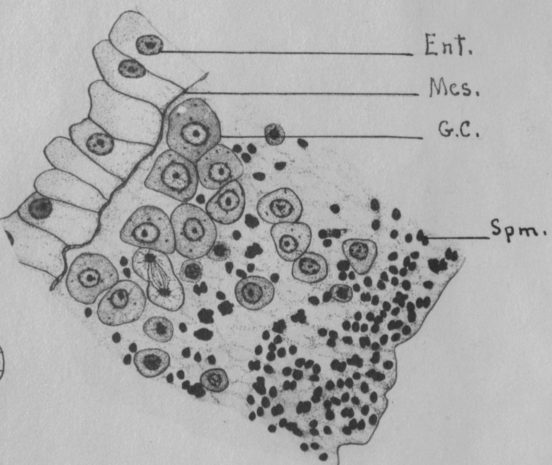

Figure 11.

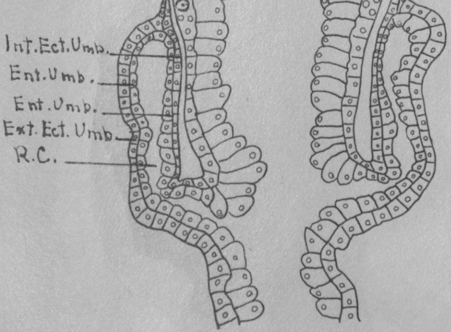

Figure 9.